

WHAT IS CLAIMED IS:

1. A method of screening *in vivo* a change in a physical, chemical, biochemical or biological, condition, the method comprising the steps of:
 - a) administering to a mammal an acceptable composition-
comprising a bioluminescent system according to claims 1 to 6;
 - b) detecting whether the light is produced; and
 - c) optionally measuring the ionic concentration of calcium flux.
2. A composition comprising a purified polypeptide, wherein said composition has the functional characteristics to binding calcium ions and to permit a measureable energy, said energy depending of the quantity of calcium bound and of the quantity of polypeptides in said composition in absence of any light excitation.
3. A purified polypeptide having the amino acid sequence of SEQ ID NO: 1.
4. A purified polypeptide having the amino acid sequence of SEQ ID NO: 2.
5. A purified polypeptide having the amino acid sequence of SEQ ID NO: 3.
6. A purified polypeptide having the amino acid sequence of SEQ ID NO: 4.
7. A purified polypeptide having the amino acid sequence of SEQ ID NO: 5.
8. A purified polypeptide having the amino acid sequence of SEQ ID NO: 6.
9. An purified polynucleotide having the sequence of SEQ ID NO: 7.
10. An purified polynucleotide having the sequence of SEQ ID NO: 8.
11. An purified polynucleotide having the sequence of SEQ ID NO: 9.
12. An purified polynucleotide having the sequence of SEQ ID NO: 10.
13. An purified polynucleotide having the sequence of SEQ ID NO: 11.
14. An purified polynucleotide having the sequence of SEQ ID NO: 12.
15. A composition according to claim 2, wherein said purified polypeptide is a purified polypeptide according to any one of claims 3 to 18.
16. A method of screening *in vitro* a change in a physical, chemical, biochemical, or biological condition, wherein the method comprises:

- (a) adding into a reaction system a composition according to claim 15 containing an analyte of interest in presence or in absence of a molecule of interest to be tested; and
- (b) visualising the emission of energy produced in step (a).

17. A method of screening of a product leading to a change in a physical, chemical, biochemical or biological condition *in vivo*, wherein the method comprises:

- (a) administering to a vertebrate a pharmaceutically acceptable medium comprising a composition according to claim 15 in presence or in absence of a molecule of interest to be tested;
- (b) detecting the energy produced in presence of said composition; and
- (c) optionally, measuring the effective concentration of said molecule of interest necessary for the detection of the energy in step (b).

18. A method of screening *in vitro* a molecule capable of modulating the energy in a composition according to claim 22, wherein the method comprises:

- (a) providing in a biological sample a composition according to claim 22 in a reaction system containing the molecule to be tested;
- (b) detecting a modulation of the energy by comparison with a control sample containing said composition according to claim 22 without the molecule to be tested; and
- (c) optionally, determining the effective minimal concentration of said molecule capable of inhibiting or increasing the energy transfer of said composition.

19. A culture containing a polynucleotide according to claim 16, said culture as deposited at the C.N.C.M. and containing the plasmid No. I-2507.

20. A culture containing a polynucleotide according to claim 17, said culture as deposited at the C.N.C.M. and containing the plasmid No. I-2508.

21. A culture containing a polynucleotide according to claim 18, said culture as deposited at the C.N.C.M. and containing the plasmid No. I-2509.

22. A culture containing a polynucleotide according to claim 20, said culture as deposited at the C.N.C.M. and containing the plasmid No. I-2510.

23. A culture containing a polynucleotide according to claim 21, said culture as deposited at the C.N.C.M. and containing the plasmid No. I-2511.
24. A culture containing a polynucleotide according to claim 22, said culture as deposited at the C.N.C.M. and containing the plasmid No. I-2512.
25. A culture containing a polynucleotide, said culture as deposited at the C.N.C.M. and containing the plasmid No. I-2513.
26. A peptide linker having the function after translation to approach a donor site to an acceptor site in optimal conditions to permit a direct transfer of energy by chemiluminescence in a purified polypeptide according to claims 3 to 8.
27. A nucleotide linker having the nucleotide sequence of SEQ ID No: 13.
28. A polynucleotide linker having the polynucleotide sequence of SEQ ID No: 14.
29. A polynucleotide linker having the polynucleotide sequence of SEQ ID No: 15.
30. A polynucleotide linker having the polynucleotide sequence of SEQ ID No: 16.
31. A polynucleotide linker having the polynucleotide sequence of SEQ ID No: 17.
32. A polynucleotide linker according to any one of claims 27 to 31 having the function after translation to approach a donor site to an acceptor site in optimal conditions to permit a direct transfer of energy by Chemiluminescence Resonance Energy Transfer (CRET) in a purified polypeptide according to claim 2.
33. A peptidic linker of at least 5 amino acids and comprising the amino acid sequence of SEQ ID No: 18.
34. A peptidic linker of at least 5 amino acids and comprising the amino acid sequence of SEQ ID No: 19.
35. A peptidic linker of at least 5 amino acids and comprising the amino acid sequence of SEQ ID No: 20.
36. A peptidic linker of at least 5 amino acids and comprising the amino acid sequence of SEQ ID No: 21.
37. A peptidic linker of at least 5 amino acids and comprising the amino acid sequence of SEQ ID No: 22.
38. A peptide linker according to any one of claims 33 to 44, having the function after translation to approach a donor site to an acceptor site in optimal

conditions to permit a direct transfer of energy in the presence of a purified polypeptide according to claim 2.

39. A peptide linker according to any one of claims 33 to 37, which has the capacity to stabilize a modified bioluminescent system *in vivo* and/or *in vitro*.

40. A modified bioluminescent system comprising two bioluminescent proteins and a peptide linker according to any one of claims 33 to 39.

41. A modified bioluminescent system according to claim 40, wherein said two bioluminescent proteins comprise at least an aequorin protein.

42. A modified bioluminescent system according to claims 40 or 41 comprising by the following constituents : aequorin protein and a GFP protein.

43. A kit for measuring the transfer of energy *in vivo* or *in vitro* and containing at least one of the polypeptides according to claims 3 to 8 or the polynucleotide according to claims 9 to 14 and the reagents necessary for visualizing or detecting the said transfer in presence or in absence of a molecule of interest.

44. A fusion protein of the formula:

GFP - LINKER - AEQ;

wherein GFP is green fluorescent protein;

AEQ is aequorin; and

LINKER is a polypeptide of 4-63 amino acids.

45. The fusion protein as claimed in claim 44, wherein the LINKER comprises 14-50 amino acids.

46. The fusion protein as claimed in claim 45, wherein the LINKER comprises the following amino acids:

(Gly Gly Ser Gly Ser Gly Gly Gln Ser [SEQ ID NO: 251])_n,

wherein n is 1-5.

47. The fusion protein as claimed in claim 46, wherein n is 1.

48. The fusion protein as claimed in claim 46, wherein n is 5.

49. The fusion protein as claimed in claim 46, wherein LINKER includes the amino acid sequence Ser Gly Leu Arg Ser [SEQ ID NO: 26].

50. A fusion protein for energy transfer from aequorin to green fluorescent protein by Chemiluminescence Resonance Energy Transfer (CRET) following activation of the aequorin in the presence of Ca⁺⁺, wherein the fusion protein has the formula:

GFP - LINKER - AEQ;

wherein GFP is green fluorescent protein;

AEQ is aequorin; and

LINKER comprises the following amino acids:

(Gly Gly Ser Gly Ser Gly Gly Gln Ser [SEQ ID NO: 25])_n,

wherein n is 1-5; and

wherein the fusion protein has an affinity for Ca⁺⁺ ions and a half-life of at least 24 hours.

51. The fusion protein as claimed in claim 50, wherein LINKER includes the amino acid sequence Ser Gly Leu Arg Ser [SEQ ID NO: 26].

52. The fusion protein as claimed in claim 50, which further comprises a peptide signal sequence for targeting the fusion protein to a cell or to a subcellular compartment.

53. A polynucleotide encoding a fusion protein as claimed in any one of claims 44 to 52.